HIV

HIV serodiscordant sex partners and the prevalence of human herpesvirus 8 infection among HIV negative men who have sex with men: baseline data from the EXPLORE Study

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Objectives: Human herpesvirus 8 (HHV-8) infection is common among men who have sex with men (MSM), especially those infected with HIV, and is frequently detected in saliva. We sought to determine whether oral or anogenital contact with HIV discordant, or unknown serostatus sexual partners is associated with HHV-8 seroprevalence among HIV negative MSM.

Methods: HIV negative MSM participating in a behavioural intervention trial for the prevention of HIV infection (the EXPLORE study) were recruited from the Seattle and Denver areas for participation in this cross sectional study. Participants completed detailed questionnaires regarding sexual behaviour, focusing on activities with possible exposure to the oropharynx. Serum samples from study enrolment were tested for the presence of HHV-8 antibodies using whole virus enzyme immunoassay and immunofluorescence assay to latent and lytic proteins.

Results: 198/819 MSM (24.3%) were HHV-8 antibody positive. Exposure to saliva with HIV positive and HIV unknown serostatus sex partners was reported by 83% and 90% of all men, respectively. In a multivariate model, reporting more than the median number of lifetime sex partners (OR 2.2, p = 0.03) or lifetime sex partners of unknown HIV status (OR 1.7, p = 0.03), and the performance of oro-anal sex (''rimming'') on partners whose HIV status is unknown (OR 2.7, p = 0.04) were independently associated with HHV-8 infection.

Conclusions: The oropharynx may be an important anatomical site in HHV-8 acquisition, and contact with HIV serodiscordant or unknown sex partners is associated with higher HHV-8 seroprevalence among HIV negative MSM.

Approximately one quarter of HIV negative men who have sex with men (MSM) and over one half of HIV positive MSM have serological evidence of HHV-8 infection,[1] the aetiological agent of Kaposi sarcoma (KS).[2] Epidemiological investigations suggest a sexual route of acquisition, but no behaviours have been consistently associated with prevalent or incident HHV-8 infection.[3] Correlates of HHV-8 infection (seropositivity or KS) include oro-anal sex,[4 5] anal sex,[4 6–9] oro-genital sex,[4] high number of sexual partners,[4 6 7] co-infection with hepatitis or gonorrhoea,[5 6] the use of inhaled nitrates,[4 10] and exposure to sexual partners known to be infected with HIV.[8 11 12]

The oropharynx may be important in HHV-8 transmission and possibly acquisition. HHV-8 DNA is found most frequently in the oropharynx; oral epithelial cells are sites of HHV-8 replication, and ''deep kissing'' (exposure to saliva) is associated with HHV-8 seropositivity.[10 12–18]

We hypothesised: (1) oropharyngeal exposure during sex may be an important mode of HHV-8 acquisition, and (2) the practice of sexual behaviours with HIV positive partners, or partners whose HIV infection status was unknown, may serve as predictors of HHV-8 infection. To examine these associations, we studied the relation between behavioural practices and HHV-8 serological status among 800 HIV negative high risk MSM.

METHODS

Study cohort

HIV negative MSM were recruited from the Seattle and Denver areas as part of the HIV Prevention Trials Network EXPLORE study, a behavioural counselling intervention designed to reduce the seroincidence of HIV infection.[19] MSM who were ⩾18 years of age, who tested HIV negative, who engaged in anal sex in the previous 12 months, and who were not in a mutually monogamous relationship were eligible for study entry. Information was obtained regarding basic demographics, sexual behaviour, and clinical symptoms (including constitutional, oropharyngeal, gastrointestinal, rheumatic, and neurological) in the previous 6 months. Participants in Seattle and Denver were given the option to enrol in an HHV-8 ancillary study, which was approved by the institutional review boards at the University of Washington and University of Colorado.

After obtaining written informed consent, participants completed a self administered questionnaire at study entry. Participants were asked to quantify the number of sexual partners they have had over both their lifetime and also the past 6 months, stratified by partners' HIV infection status (positive, negative, or unknown). Questions regarding the practice of specific sexual behaviours involving exposure to oral, anal, and urethral secretions were asked in relation to the percentage of HIV positive, negative, and unknown partners with whom the behaviour was practised. Categorical responses were recorded (behaviour practised with 0–25%, 26–50%, 51–75%, or 76–100% of partners) for lifetime

Abbreviations: EIA, enzyme immunoassay; GEE, generalised estimating equations; HHV-8, human herpesvirus 8; IFA, immunofluorescence assay; IQR, interquartile range; KS, Kaposi sarcoma; MSM, men who have sex with men

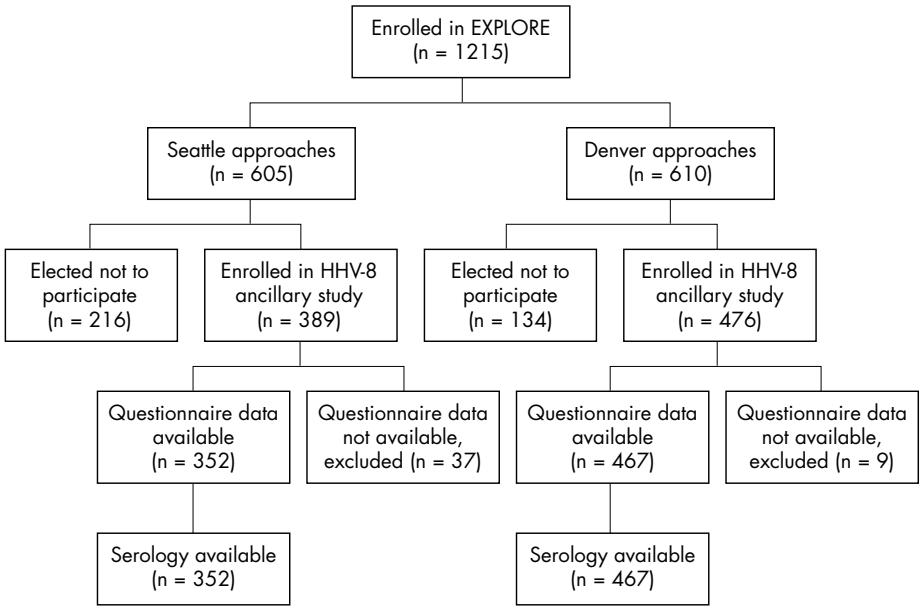

Figure 1 Flow diagram of study.

behaviours, and as integers for behaviours during the last 6 months (that is, number of partners and number of days over the last month that a particular behaviour was practised). Only participants with data from the additional oral questionnaire were included.

Serological methods

HHV-8 serological testing was performed at the initial study visit using a whole virus enzyme immunoassay (EIA) with latent and lytic antigen immunofluorescence assay (IFA) confirmation of selected samples.[20]

Data analysis

Information from questionnaires was entered into an electronic database (Microsoft Access 2000, Redmond, WA, USA) and abstracted to a statistical analysis package (Intercooled Stata Version 8, College Station, TX, USA). Descriptive statistics were used to summarise data using rates, proportions, and graphic displays. T tests were used to compare means and χ^2 tests were used to compare proportions, and the Mantel-Haenszel test for trend compared distributions across ordered categorical variables. Behavioural data were modelled dichotomously, categorically, or continuously where appropriate.

To explore the possibility that participants modified their sexual behaviour according to the HIV status of their sex partner(s), we used generalised estimating equations (GEE). We estimated the odds of engaging in each behaviour with >25% of HIV negative partners as a reference category, and compared that to the odds of the same behaviour with >25% of HIV positive or HIV unknown partners, using indicators for partner type.

To determine behavioural correlates of HHV-8 infection, we computed the total number of partners of each HIV serostatus with whom the behaviour was practised, as each additional partner would potentially affect the odds of having acquired HHV-8. Each behaviour queried over the lifetime was dichotomised at $\leqslant 5$ versus >5 partners, while each 6 month history behaviour was summarised as either ever practised or not. Number of lifetime partners was dichotomised at the median value, since responses varied widely by type of partner. Lastly, to determine whether behaviours practised with HIV positive individuals were associated with an increased risk of HHV-8, each behaviour was modelled using groups of three indicators: above cutoff with (1) partners of any status, (2) HIV positive partners, and (3) HIV unknown partners. Logistic regression, both univariate and multivariate, was then used to examine behaviours associated with odds of HHV-8 positivity. A multivariate model was selected using backward elimination with inclusion of factors significant at 0.10 univariately.

RESULTS

Study participants

A total of 1215 participants enrolled in the HPTN EXPLORE trial at the Seattle and Denver sites (fig 1), of whom 389 of 605 (64.2%) Seattle participants and 476 of 610 (78.0%) in Denver consented to participate in the HHV-8 ancillary study. Participants who declined entry into the ancillary study were more likely to have been seen at the Seattle study site (odds ratio (OR) 1.3, 95% confidence interval (CI) 1.1 to 1.4, $p<0.001$), were slightly older (mean age 1.4 years older, 95% CI 0.4 to 2.4, $p = 0.01$), had less formal education (91.0% continued education beyond high school in the enrolled cohort versus 82.7% of people who declined enrolment, $p = 0.006$), and were more likely to be people of racial or ethnic minorities (15% of the enrolled cohort versus 20.6% of people who declined enrolment, $p<0.001$). There were no significant differences in the number of HIV positive sex partners ($p = 0.24$), HIV negative sex partners ($p = 0.15$), or HIV unknown sex partners ($p = 0.28$) between those who enrolled and declined participation in the HHV-8 ancillary study. Questionnaires were available for 352 of 389 (90.5%) Seattle participants and 467 of 476 (98.1%) participants from Denver. The demographics of the study participants are shown in table 1.

There was no significant difference in income between people who differed in enrolling study site, but the mean age of participants in Denver was 1.5 years older than those in Seattle ($p = 0.03$). Participants in Seattle were more likely to report their race as white (89.2% *v* 82.4%) or Asian/Pacific Islander (4.3% *v* 1.0%) compared with participants in Denver, while more Denver enrollees identified their race as black (3.4% *v* 1.7%). At enrolment, 198 of 819 (24.2%) participants were HHV-8 seropositive. There were no significant differences in HHV-8 serostatus by race, income, or study site.

Table 1 Demographics of study cohort by HHV-8 serostatus

	Total	HHV-8 seronegative	HHV-8 seropositive	p Value
Number of participants (%)	819	621 (75.8)	198 (24.2)	
Study site				
Denver (%)	467	355 (76.1)	112 (23.9)	0.89*
Seattle (%)	352	266 (75.6)	86 (24.4)	
Age (median, years)	34.3	34.2	34.7	0.45
Race				
White	85.5	84.5	87.9	
Black	2.7	2.4	3.5	
Asian/Pacific Islander	2.4	2.8	1.5	0.29†
Native American	1.2	1.3	1.0	
Other	8.3	9.1	6.1	
Education				
Less than high school	1.3	1.3	1.5	
High school degree	7.7	8.1	6.6	
Some college	32.1	31.1	35.4	
College degree	33.8	33.7	34.3	0.38†
Some graduate work	7.9	7.9	8.1	
Graduate degree	17.1	18.0	14.1	
Income (yearly)				
<$6000	4.8	4.7	5.1	
$6000–11 999	6.4	6.5	6.1	
$12 000–29 999	31.1	28.9	37.4	0.16†
$30 000–59 999	40.2	42.1	34.3	
>$60 000	17.6	17.9	17.2	

*Denver *v* Seattle HHV-8 seropositive. †Mantel-Haenzel test for trend between HHV-8 seronegative and seropositive.

Practice of sexual behaviours by the study cohort

All evaluated sexual practice behaviours are shown in table 2. The number of lifetime sex partners varied by the partner's HIV status, with a median of one known HIV positive partner (interquartile range (IQR) 0–5), 30 HIV negative partners (IQR 9–100), and 50 lifetime partners whose HIV status was unknown (IQR 10–200).

With the exception of protected oral sex, all sexual behaviours surveyed were reported as more commonly ever performed with HIV negative or HIV unknown partners compared with HIV positive partners ($p<0.01$), suggesting that participants modified their sexual behaviours based on perception of risk of infection with HIV. Additionally, high risk and unprotected sexual behaviours were practised with a similar proportion of HIV negative and unknown partners but a lower proportion of HIV positive partners, further suggesting that participants perceived the risk of infection from HIV unknown partners as similar to the risk of infection from HIV negative partners. The routine use of condoms (defined as ⩾25% of partners) was less common with oral sex compared with other sexual practices.

Practice of sexual behaviours with exposure to saliva

Deep kissing in the previous 6 months was common, reported by 75.3% of the cohort, and practised with similar proportions of HIV unknown and HIV negative partners (OR 1.0, $p = 0.99$). However, deep kissing was over half as likely to be reported with HIV positive partners (OR 0.46, $p<0.001$). Approximately 45% of respondents reported deep kissing more than three people on a given night at a bar, party, or other social setting during the past 6 months, as a potential surrogate indicator of heightened risk for exposure to saliva from multiple partners. Exposure to saliva through lubrication during anal sex, receipt of rimming, or oral sex was also common.

Predictors of HHV-8 seropositivity

Sexual behaviours

Among all sex partners, a significant relation was observed between HHV-8 serostatus and reporting more than the median number of lifetime sex partners (OR 2.5, 95% CI 1.2 to 5.0, $p = 0.01$) (table 3). The only other factor, which was associated with HHV-8 infection when all sex partners were considered in aggregate, was the frequent use of condoms while receiving anal sex (OR 0.5, 95% CI 0.3 to 1.0 $p = 0.04$). No relation was found between HHV-8 infection and the total number of HIV negative partners, or any sexual behaviour practised only with HIV negative partners.

Although reporting more than the median number of HIV positive lifetime sex partners was not significantly associated with HHV-8 infection (OR 1.7, 95% CI 1.1 to 2.6, $p = 0.02$), three specific sexual practices performed with HIV positive partners were unprotected insertive oral sex without ejaculation (OR 2.0, 95% CI 1.1 to 3.4, $p = 0.02$), unprotected insertive anal sex (OR 10.8, 95% CI 1.1 to 107, $p = 0.04$), and deep kissing (OR 1.8, 95% CI 1.1 to 3.0, $p = 0.02$).

In contrast, HHV-8 infection was more common in people reporting more than the median number of HIV unknown sex partners (OR 1.7, 95% CI 1.1 to 2.6, $p = 0.02$). The performance of unprotected oral sex leading to ejaculation (OR 2.2, 95% CI 1.1 to 4.3, $p = 0.02$), protected receptive anal sex (OR 2.3, 95% CI 1.2 to 4.2, $p = 0.01$), unprotected insertive anal sex (OR 2.6, 95% CI 1.1 to 6.1, $p = 0.02$), and rimming (OR 3.3, 95% CI 1.3 to 8.5, $p = 0.01$) were all associated with HHV-8.

As there are a number of ways to parameterise the degree of participation in behaviours with partners of different HIV infection statuses, we performed a second analysis examining the proportion of HIV unknown and HIV positive partners with which each behaviour was performed who were of HIV unknown and HIV positive serostatus. The results of this additional parameterisation agreed with the first in that behaviours with HIV positive or HIV unknown partners were the only ones significantly associated with HHV-8 infection. The specific behaviours associated with HHV-8 infection agreed in the two analyses, with three exceptions: oral insertive sex and receipt of unprotected anal sex was not significantly associated with HHV-8 infection in the second analysis, and deep kissing in the last 6 months was.

Although the number of participants reporting a history of pharyngeal gonorrhoea or rectal sores was small, each was found to be associated with HHV-8 infection (1/618 HHV-8 negative participants reported pharyngeal gonorrhea *v* 3/198 HHV positive men, OR 9.5, 95% CI 1.0 to 91.5, $p = 0.05$, and

Table 2 Sexual behaviour among study cohort by partners' HIV status

	HIV status of partner(s)							
	Negative		Unknown		Positive		Any	
Behaviour	n/total* (%)	OR, p value	n/total* (%)	OR† p value	n/total* (%)	OR‡ p value	n/total* (%)	OR, p value
Lifetime sexual behaviours								
Number of lifetime partners by HIV status	564/589 (96)	ref	533/589 (90)	ND	389/589 (66)	ND	589/589 (100)	ND
Performance of insertive oral sex								
Partner wearing condom	108/555 (19)	ref	97/527 (18)	0.94, 0.6057	85/367 (23)	1.36, 0.0256	205/589 (35)	ND
Partner not wearing condom, with ejaculation	253/537 (47)	ref	152/523 (29)	0.46, <0.0001	45/362 (12)	0.16, <0.0001	300/589 (51)	ND
Partner not wearing condom, no ejaculation	425/494 (86)	ref	418/517 (81)	0.67, 0.0044	190/356 (53)	0.18, <0.0001	520/585 (89)	ND
Receipt of anal sex								
Partner wearing condom	348/545 (64)	ref	270/525 (51)	0.59, <0.0001	136/364 (37)	0.37, <0.0001	403/587 (69)	ND
Partner not wearing condom	160/539 (30)	ref	91/528 (17)	0.49, <0.0001	27/361 (7)	0.17, <0.0001	194/588 (33)	ND
Performance of insertive anal sex								
While wearing condom	377/549 (69)	ref	315/526 (60)	0.67, <0.0001	173/360 (48)	0.41, <0.0001	439/587(75)	ND
Not wearing condom	199/543 (37)	ref	141/525 (27)	0.61, <0.0001	76/363 (21)	0.38, <0.0001	250/588 (43)	ND
Receipt of oral sex								
While wearing condom	94/533 (18)	ref	74/516 (14)	0.82, 0.1246	59/355 (17)	1.00, 0.9751	166/585 (28)	ND
Not wearing condom	491/530 (93)	ref	476/526 (90)	0.74, 0.1234	266/362 (74)	0.21, <0.0001	563/587 (96)	ND
Performed rimming	224/556 (40)	ref	154/525 (29)	0.60, <0.0001	75/365 (21)	0.35, <0.0001	268/589 (46)	ND
Deep kissing in lifetime	528/556 (95)	ref	447/529 (90)	0.48, <0.0001	303/366 (83)	0.25, <0.0001	565/589 (96)	ND
Sexual behaviours in past 6 months								
Deep kissing in past 6 months	603/693 (87)	ref	522/692 (75)	1.00, 0.9938	181/306 (59)	0.46, <0.0001	749(819)91	ND
Number of partners, past 6 months, by HIV status	NA¶	–	NA¶	–	207/819 (25)	ND	810/819 (99)	ND
Use of saliva as lubricant to anal sex	NA¶	–	NA¶	–	40/244 (16)	0.28, <0.0001	313/809 (39)	ref
Received oro-anal sex (''rimming'')	NA¶	–	NA¶	–	89/251 (35)	0.24, <0.0001	541/812 (67)	ref

*Proportion of men reporting each sexual behaviour with >25% partners of given HIV status among participants with ⩾1 HIV negative sex partner. †Odds of performing each sexual behaviour with >25% of HIV status unknown sex partners compared with >25% of HIV negative sex partners, among participants reporting ⩾1 HIV unknown sex partner. ‡Odds of performing each sexual behaviour with >25% of HIV positive sex partners compared with >25% of HIV negative sex partners, among participants reporting ⩾1 HIV positive sex partner. ‡Odds of performing each sexual behaviour with >25% of HIV positive sex partners compared with >25% of any sex partners, among participants reporting ⩾1 HIV positive sex partner. ¶These questions were not asked of HIV negative partners. ND, this calculation not done.

Table 3 Univariate predictors of HHV-8 serostatus among HIV negative MSM

	HIV positive			HIV status unknown			Any status		
	HHV-8−	HHV-8+		HHV-8−	HHV-8+		HHV-8−	HHV-8+	
	N/total (%)	N/total (%)	OR* (95% CI)	N/total (%)	N/total (%)	OR† (95% CI)	N/total (%)	N/total (%)	OR‡ (95% CI)
Lifetime sexual behaviours									
>Median lifetime sex partners¶	195/435 (45)	93/152 (61)	1.2 (0.7 to 1.7)	177/435 (41)	97/152 (64)	**1.7 (1.1 to 2.6)**	293/435 (67)	135/152 (89)	**2.5 (1.2 to 5.0)**
Performance of insertive oral sex									
Partner wearing condom	21/422 (5)	6/143 (4)	1.1(0.4 to 3.1)	39/431 (9)	12/150 (8)	1.3 (0.5 to 3.3)	84/435 (19)	24/152 (16)	0.7 (0.3 to 1.6)
Partner not wearing condom, plus ejaculation	5/417 (1)	7/143 (5)	3.1 (0.9 to 10.6)	52/427 (12)	34/150 (23)	**2.2 (1.1 to 4.3)**	131/435 (30)	59/152 (39)	1.0 (0.6 to 1.5)
Partner not wearing condom, minus ejaculation	40/416 (10)	25/138 (18)	2.0 (1.1 to 3.4)	237/427 (56)	94/144 (65)	1.3 (0.7 to 2.1)	325/434 (75)	120/149 (81)	1.2 (0.7 to 2.3)
Receipt of anal sex									
Partner wearing condom	22/419 (5)	13/143 (9)	1.9 (0.9 to 4.1)	111/430 (26)	52/149 (35)	**2.3 (1.2 to 4.2)**	203/433 (47)	71/152 (47)	**0.5 (0.3 to 1.0)**
Partner not wearing condom	1/418 (0)	4/141 (3)	**10.8 (1.1 to 106.7)**	33/433 (8)	18/149 (12)	2.6 (0.9 to 7.2)	69/434 (16)	28/152 (18)	0.6 (0.2 to 1.3)
Performance of insertive anal sex									
While wearing condom	35/417 (8)	16/141 (11)	1.4 (0.7 to 2.8)	127/431 (29)	51/149 (34)	1.5 (0.8 to 2.7)	208/433 (48)	74/152 (49)	0.8 (0.4 to 1.3)
Not wearing condom	12/419 (3)	10/142 (7)	2.2 (0.8 to 5.7)	41/431 (10)	27/148 (18)	**2.6 (1.1 to 6.1)**	87/434 (20)	40/152 (26)	0.7 (0.3 to 1.5)
Performed rimming	6/421 (1)	4/142 (3)	1.5 (0.4 to 5.9)	42/430 (10)	26/149 (17)	**3.3 (1.3 to 8.5)**	82/435 (19)	34/152 (22)	0.6 (0.3 to 1.4)
Deep kissing over lifetime	66/421 (16)	38/143 (27)	1.8 (1.1 to 3.0)**	261/434 (60)	104/149 (70)	1.5 (0.9 to 2.6)	362/435 (83)	128/152 (84)	0.7 (0.3 to 1.4)
Receipt of oral sex									
While wearing condom	10/414 (2)	6/139 (4)	3.0 (0.9 to 9.9)	27/421 (6)	7/149 (5)	1.2 (0.4 to 3.8)	68/432 (16)	21/151 (14)	0.5 (0.2 to 1.3)
Not wearing condom	69/419 (16)	35/141 (25)	1.5 (1.0 to 2.5)	276/430 (64)	110/150 (73)	1.4 (0.8 to 2.3)	365/434 (84)	135/151 (89)	1.3 (0.6 to 2.7)
Sexual behaviours in last 6 months									
Deep kissing over last 6 months§	127/602 (21)	54/195 (28)	1.5 (1.0 to 2.1)	385/598 (64)	130/197 (66)	1.1 (0.8 to 1.6)	560/617 (91)	180/198 (91)	0.8 (0.4 to 1.6)
Use of saliva as lubricant to anal sex§	29/588 (5)	11/189 (6)	1.0 (0.5 to 2.1)	to	to	to	222/612 (36)	84/197 (43)	1.3 (0.9 to 1.9)
Received oro-anal sex ("rimming")§	62/580 (11)	26/182 (14)	1.3 (0.8 to 2.2)	to	–	–	392/610 (64)	137/196 (70)	1.2 (0.8 to 1.7)

For most behaviours, numbers and percentages indicate those who engaged in this behaviour with at least five partners of that type (exceptions are below). All subjects who perform the behaviour with one or more partners of any type are included in each analysis, which compares simultaneously the rates of these behaviours between HIV negative, HIV unknown and HIV positive partners. Each row represents a single model, where odds ratios are adjusted for behaviours with partners of other HIV status within that row.

p Values, odds ratios in bold $p<0.05$, underlined $p>0.05$ but <0.1.

*Odds ratio is relative to participants engaging in this behaviour with at least 5 partners but not 5 HIV positive partners.

†Odds ratio is relative to participants engaging in this behaviour with at least 5 partners but not 5 HIV unknown partners.

‡Odds ratio is relative to participants engaging in this behaviour with less than 5 partners of any type.

¶Proportion with > median number of partners in any category in lifetime: 1 for HIV positive, 30 for HIV negative, and 50 for HIV unknown partners.

§Proportion engaging in behaviour at least once in the last 6 months.

10/618 HHV-8 negative participants reported rectal sores *v* 8/198 HHV-8 positive participants, OR 2.6, 95% CI 1.0 to 6.6, p = 0.05).

Popper (amyl nitrate) use was also significantly associated with HHV-8 infection. Compared with men who never used ''poppers,'' use less than once a week was associated with a 1.3-fold increased odds of HHV-8 infection (95% CI 0.9 to 1.9, p = 0.14), 1–2 times per week 2.9-fold increase (95% CI 1.6 to 5.4, p<0.01), and ⩾3 days per week 3.4-fold increase (95% CI 1.4 to 8.6, p = 0.01). However, popper use was also significantly associated with the median number of sex partners of any HIV status, having more than the median number of HIV unknown sex partners, and performing rimming with >5 lifetime HIV unknown sex partners (p<0.001).

In a multivariate model, three factors were independently associated with HHV-8 infection at baseline (table 4): reporting more than median number of lifetime sex partners (OR 2.2, 95% CI 1.1 to 4.6, p = 0.03) or less than median number of lifetime sex partners of unknown HIV status (OR 1.7, 95% CI 1.1 to 2.7, p = 0.03), or the performance of rimming on partners whose HIV status is unknown (OR 2.7, 95% CI 1.0 to 7.1, p = 0.04).

DISCUSSION

Guided by previous virological and epidemiological research suggesting that exposure to saliva could be an important factor in the acquisition of HHV-8 infection, that the oropharynx is a site of active HHV-8 replication, and that the prevalence of HHV-8 is greatest among HIV positive MSM,[10 12–18] we conducted the first large study which comprehensively surveyed behaviours associated with exposure to the oropharynx, stratified by sex partner's HIV infection status, and looked for associations with prevalent HHV-8 infection.

Our study is among the first to extensively characterise the extent to which sexual behaviours with exposure to saliva are practised among MSM in the United States. We found that deep kissing, oro-penile, and oro-anal contact are all commonly practised with both HIV positive and uninfected men, and frequently is unprotected. Only one previous study has specifically measured exposure to saliva and examined its relation to HHV-8 infection.[12] This study found that a sevenfold higher odds of HHV-8 seropositivity among MSM who engaged in deep kissing, and noted that HHV-8 DNA was detected in oropharyngeal swabs from over one third of HHV-8 seropositive men on over one third of the days that oropharyngeal samples were collected. This study, however, did not inquire about exposure to saliva other than deep kissing. Virological data suggest that the oral cavity of a person infected with HHV-8, where infectious virus is shed,[17] may be a source of transmissible HHV-8. The near ubiquitous practice of behaviours associated with exposure to saliva made it difficult to calculate their contribution to HHV-8 infection. Future virological and behavioural studies are needed to determine whether exposure to saliva is associated with acquisition of HHV-8 infection.

HHV-8 DNA has been detected less frequently at ano-genital sites than in saliva in previous studies,[21] but it is possible that exposure to ano-genital shedding may also result in HHV-8 transmission. The oropharynx has been hypothesised to be the site of primary infection,[22] and therefore the exposure of the oropharynx to HHV-8 shed in the ano-genital mucosa during oro-anal sex could result in its acquisition. Studies are needed to determine whether HHV-8 shed at rectal sites includes infectious virions and whether such shedding is associated with transmission of HHV-8.

The strong and dose dependent relation between popper use and HHV-8 infection has been found consistently in epidemiologic studies. A number of explanations have been offered for this association, ranging from the effects of nitrates on immune function to the vasodilatory properties of these drugs.[23 24] We found evidence for amyl nitrate use being significantly associated with sexual behaviours that could confer a greater risk of STI acquisition. Therefore the question of whether the relation between amyl nitrates and HHV-8 is biological or behavioural remains unanswered.

Sexual behaviour varied dramatically according to the HIV infection status of the sex partner in this cohort. Similarly, the magnitude of the association between HHV-8 seropositivity and these sexual behaviours varied according to the sex partner's HIV status. Previous studies have shown that HIV positive MSM are nearly twice as likely to be infected with HHV-8 as HIV negative MSM,[25 26] and reporting at least one recent HIV positive partner is associated with a greater risk of HHV-8 infection.[11 12] It is unclear whether the increased prevalence reflects a biological interaction between HHV-8 and HIV, or the potential for common risk factors to predispose to both infections. Oropharyngeal HHV-8 shedding may also be directly proportional to HIV plasma RNA level.[27] The infrequent reporting of known HIV positive sexual partners, coupled with the tendency to practise less risky sex with HIV positive partners may have mitigated our ability to detect an association between HIV positive partners and HHV-8 infection. The significant association of HHV-8 seropositivity with partners of unknown HIV status, however, is probably because of exposure to HIV positive men, given HIV prevalence of approximately 15% among MSM in Denver and Seattle, as well as ''riskier'' sexual behaviours with partners whose HIV status was not known compared with known HIV positive partners.

This study had several important limitations. Firstly, the data presented here represent a cross sectional analysis of baseline information and the date of HHV-8 acquisition is not known, thus a temporal relation between significant risk factors and HHV-8 infection cannot be established. Retrospective reporting of sexual behaviour history and misclassification, particularly of lifetime sexual behaviours, is subject to recall bias. Future analyses of these high risk HHV-8 negative MSM will allow for associations to be made

Table 4 Multivariate model of predictors of HHV-8 seropositivity

Predictor	Odds ratio	95% CI	p Value
More than median no of partners in lifetime			
Any partners*	2.2	1.1 to 4.6	0.03
HIV+ partners	1.1	0.7 to 1.8	0.63
HIV status unknown partners	1.7	1.1 to 2.7	0.03
Performed rimming on at least five partners			
Partners of any status	0.6	0.2 to 1.4	0.21
HIV+ partners	1.4	0.4 to 5.6	0.64
HIV status unknown partners	2.7	1.0 to 7.1	0.04

*Measures proportion with more than median number of partners in any category in lifetime: 1 for HIV+, 30 for HIV− and 50 for HIV unknown partners.

between prospectively collected behavioural data and newly acquired HHV-8 infection. Additionally, sexual behaviours with exposure to saliva were so widely practised by all participants in this study (>50%) that the power of an epidemiological investigation to find an association between exposure to saliva and HHV-8 serostatus is limited. As shown in table 2, the majority of participants reported practising both anogenital sex and deep kissing with sex partners of every HIV status, making it difficult to disentangle the highest risk behaviours. The dichotomisation scheme we chose to analyse sexual behaviours does not allow the quantification of risk per additional sex partner, by allowing for the capture of large effects in a conservative manner. This study was also limited by the lack of any information on the severity of HIV disease or treatment regimens of HIV positive sex partners. We have recently found that HAART may decrease HHV-8 oropharyngeal shedding and, therefore, could have a significant impact on the transmissibility of HHV-8 from HIV positive sex partners.[28] Finally, as mentioned above, it is not possible to determine whether those behaviours found to be significantly associated with HHV-8 infection were surrogates for extremely ''risky'' sexual contacts, among whom HHV-8 prevalence is likely to be higher. Analysis of incident HHV-8 cases will allow for partner specific sexual behaviour to be analysed, and should provide the opportunity to examine the relation between the types of sexual behaviour which may predispose to acquisition of HHV-8.

Despite advances in the characterisation of the basic virology and epidemiology of HHV-8 infection, significant questions remain regarding transmission and acquisition. The confusing observation that HHV-8 appears to be acquired before the onset of puberty in endemic areas but behaves more like a sexually transmitted pathogen among MSM may be reconciled by finding a role for exposure to saliva in HHV-8 transmission. Refining the questions asked in epidemiological surveys and conducting careful studies of the mucosal sites of HHV-8 shedding may allow for a greater understanding about the modes of HHV-8 transmission and effective strategies to prevent acquisition of infection.

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